Kidney International, Vol. 35 (1989), pp. 638–646

Retroviral gp70 antigen in spontaneous mesangial glomerulonephritis of ddY mice

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Retroviral gp70 antigen in spontaneous mesangial glomerulonephritis of ddY mice. We examined whether the retroviral envelope antigen, gp70, is a major nephritogenic antigen in ddY mice, a murine model of spontaneous mesangial glomerulonephritis associated with IgA and IgG deposition. Immunofluorescence microscopy revealed that the mesangial gp70 deposition increased with age in mice over 24 weeks old, as did the IgG and IgA deposits. Immunoelectron microscopy demonstrated the reaction products of gp70 superimposed on the electron dense deposits in the mesangial matrix. Various amounts of serum gp70 were detected in mice as young as 12 weeks without any apparent increase with age. There was no correlation between the serum level of gp70 and the extent of the glomerular gp70 deposition, whereas mice with heavier IgA deposition had higher mean levels of serum IgA. The absorption test demonstrated that significant amounts of serum gp70 composed immune complexes in 40 week-old ddY mice developing glomerulonephritis; however, this bound form of gp70 was not observed in 12 week-old mice without glomerulonephritis. Systemic examinations by immunofluorescence staining showed that gp70 was mainly localized in various lymphoid tissues. These findings suggest that the gp70 antigen, mostly derived from lymphoid cells, may circulate as immune complexes and accumulate in the mesangial area, thus contributing to the development of glomerulonephritis in these mice. In addition, the pathogenic role of the increased IgA production in these mice was discussed.

IgA nephropathy has been described as one of the major primary glomerulonephritis, characterized by the mesangial deposition of IgA, which is often associated with IgG and C3 deposition [1–4]. Although the pathogenesis of this disease is still unclear, researchers have made various hypotheses regarding the role of circulating IgA immune complexes [5–10]. Clinical data gathered to date suggest that factors such as upper respiratory infections [11, 12], dietary antigens [13] and hereditary backgrounds [14–17] may contribute to the hypersecretion of IgA, which is the major immunological hallmark of this disease [18–21].

The experimental murine model of IgA nephropathy described by Rifai et al, induced by the dinitrophenol(DNP)-IgA anti-DNP antibody system demonstrated the pathogenesity of the polymeric form of IgA [22]. Regarding the antigenic study, Isaacs, Miller and Lane have shown that immunization of a polysaccharide antigen, dextran, in BALB/c mice can induce the mesangial deposition of IgA antibodies [23]. Emancipator, Gallo and Lamm demonstrated that oral immunization of protein antigens in mice elicited polymeric IgA response producing mesangial IgA deposits, suggesting the pathogenic importance of dietary antigens as well as the mucosal IgA antibody response [24]. On the other hand, in a study using a mink model affected with Aleutian disease, IgA was found to be the predominant deposit in the diseased glomeruli, and the pathogenic role of viral antigen-antibody complexes was suggested [25, 26].

Recently, in search of a spontaneous murine model of IgA nephropathy, Imai et al observed that the ddY mice developed mesangial proliferative glomerulonephritis later in life, associated with prominent mesangial IgA deposition [27]. On the other hand, Chino, Sato and Sasaki have already reported that retroviral infections are related to high incidences of malignant tumors in these mice [28].

In this report, we studied the possible role of a retroviral envelope glycoprotein, gp70, in the pathogenesis of glomerulonephritis in ddY mice. Previous researches have established that gp70 is one of the major nephritogenic antigens in various mice which develop lupus nephritis or necrotizing arteritis-associated glomerulonephritis, all of which bear endogenous murine leukemia virus (MuLV) infections [29–32]. The results of this study suggest that circulating gp70-anti-gp70 immune complexes may contribute to the development of mesangial proliferative glomerulonephritis in these mice.

Methods

Mice

Female and male ddY mice which were maintained as a closed colony, and BALB/c mice were purchased from Shizuoka Agricultural Cooperative Association for Laboratory Animals, Shizuoka, Japan and bred in our colony. The ddY mice were bled and sacrificed at 12, 16, 24, 32, 40, 50 and 60 weeks-old. Only female mice were used in the present study. Obtained blood samples were kept at −70°C until use.

Histological examinations

Tissue specimens from each organ were fixed in Dubosque-Brazil solution for light microscopy, and the sections were

Received for publication May 18, 1988
and in revised form September 8, 1988

stained with hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS). For immunofluorescence study, the tissue specimens embedded in mounting media (Tissue Tek O.C.T. Compound, Miles Laboratories, Inc., Naperville, Illinois, USA) were quick-frozen using dry ice-acetone. Cryostat sections were stained directly with FITC-conjugated antibodies against mouse IgM, IgG and IgA (Cappel Laboratories, Cochranville, Pennsylvania, USA). The monospecificity of these antibodies was confirmed by double immunodiffusion and immunoelectrophoresis. The detection of tissue gp70 was done using an indirect fluorescence method with goat antiserum against gp69/71 purified from Rauscher murine leukemia virus, provided by Dr. John S. Cole III, National Cancer Institute, Bethesda, Maryland, USA. The sections were subsequently incubated with FITC-conjugated anti-goat IgG antibodies (Cappel Laboratories). As a control, normal goat serum was used instead of goat anti-gp70 antiserum. The specificity of the gp70 staining was controlled by absorbing the goat anti-gp70 antiserum with gp70-rich concanavalin A fractions of sera from New Zealand mice as described below.

Ultrastructural studies of kidney specimens

Small blocks of the renal cortex were fixed in 2% glutaraldehyde and then in 1% osmic acid for electron microscopy. After being embedded in Epon 812, ultrathin sections were treated with uranyl acetate and lead citrate. In order to determine the gp70 localization by immunoelectron microscopy, the cortex specimens were fixed with periodate-lysine-paraformaldehyde solution [33]. After being incubated in solutions of 10%, 15% and 20% sucrose in phosphate-buffered saline (PBS), the specimens were embedded in the mounting media, frozen, then cut at 8 μm thickness. The IgG fractions of goat anti-gp70 antiserum were prepared with DEAE agarose gel coupled with Cibacron Blue dye (DEAE Affi-Gel Blue, Bio-Rad, Richmond, California, USA), and labeled with biotin [34]. The sections were reacted with biotin-labeled anti-gp70 antibodies and then with horseradish peroxidase-avidin D complex solution (Vector Laboratories, Inc., Burlingame, California, USA), followed by fixation in 2% glutaraldehyde. The color was developed using 3,3'-diaminobenzidine as a substrate. The sections were post-fixed in 1% osmic acid solution, and then embedded in Epon 812. Ultrathin sections were prepared for electron microscopic observation. To distinguish the localization of the immunoreaction products from that of electron dense deposits, some sections were poststained with uranyl acetate.

Preparation of gp70 fraction

Pooled sera from New Zealand mice, obtained after lipopolysaccharide injection, which contain high gp70 activity [35], were subjected to the column chromatography with concanavalin A (ConA)-coupled agarose gel (ConA-Sepharose 4B, Pharmacia Fine Chemicals, Uppsala, Sweden). The ConA-bound glycoproteins were further purified using immunoaffinity agarose gel column prepared with Tresyl-activated Sepharose 4B (Pharmacia Fine Chemicals) coupled with an IgG fraction of goat anti-Rauscher gp70 antiserum [36]. The purity of gp70 was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis [36].

Measurement of serum gp70 activity

Serum gp70 levels were measured by inhibition radioimmunoassay [31]. A mixture of 50 μl of 1:2.5 diluted serum samples and 20 μl of diluted goat anti-Rauscher gp70 antiserum was incubated at 4°C overnight. Then, 20 μl of ^{125}I-labeled gp70 was added to this mixture. After further incubation at 4°C overnight, 100 μl of diluted rabbit anti-goat IgG antiserum (MBL, Nagoya, Japan) was added to each tube. The reaction mixture was incubated for two hours at room temperature, and then for one hour at 4°C. The resulting immunoprecipitates were washed by centrifugation, and the radioactivity was measured using a gamma counter. After the background correction, the results were expressed in units, according to a standard curve obtained with serially-diluted pooled sera from aged New Zealand Black (NZB) mice. The gp70 content in the NZB sera diluted to 1:2.5 was tentatively defined as 100 units.

Measurements of serum immunoglobulins

Serum concentrations of IgM, IgG and IgA were measured by solid-phase enzyme-linked immunosorbent assays [37]. Briefly, microtiter plates were coated with goat IgG antibodies against mouse IgM, IgG or IgA antibodies (Cappel Laboratories). After diluted serum samples were reacted with the plates, alkaline phosphatase-labeled antibodies to each immunoglobulin class (Cappel laboratories) were reacted to the bound proteins. The results were expressed according to the standard curve obtained by serially diluted immunoglobulins of each class.

Absorption test of serum gp70 activities

To determine whether gp70 binds to IgG or IgA antibodies in sera, 0.5 ml of each of the two serum pools from 12 week-old and 40 week-old ddY mice was passed through 1 ml of goat anti-IgG or anti-IgA antibody-coupled agarose gel column prepared with tresyl-activated Sepharose 4B (Pharmacia Fine Chemicals). A bovine serum albumin (BSA)-coupled agarose gel column was used as a control. After the sera were passed through each of these columns, concentrations of IgG, IgA and gp70 in the effluent fractions were measured as described above. The percent ratio of each protein in the total effluent fractions after absorption with each anti-immunoglobulin column to that after passing through the BSA column, was calculated.

Statistical methods

Statistical comparisons were determined using Student's *t*-test for unpaired data.

Results

Glomerular immunopathology

Diffuse mesangial cell proliferation was initially observed in 24 week-old mice which had increased PAS-positive materials in the mesangial area, using a light microscope. These findings were much more obvious in mice over 40 weeks old (Fig. 1A). Various degrees of mesangial deposition of gp70, IgM, IgG and IgA were observed in mice over 16 weeks old by immunofluorescence. Three out of 20 mice aged 50 to 60 weeks exhibited crescentic glomerulonephritis with intensive mesangial deposition of these proteins (Fig. 1B). The characteristic IgA deposi-

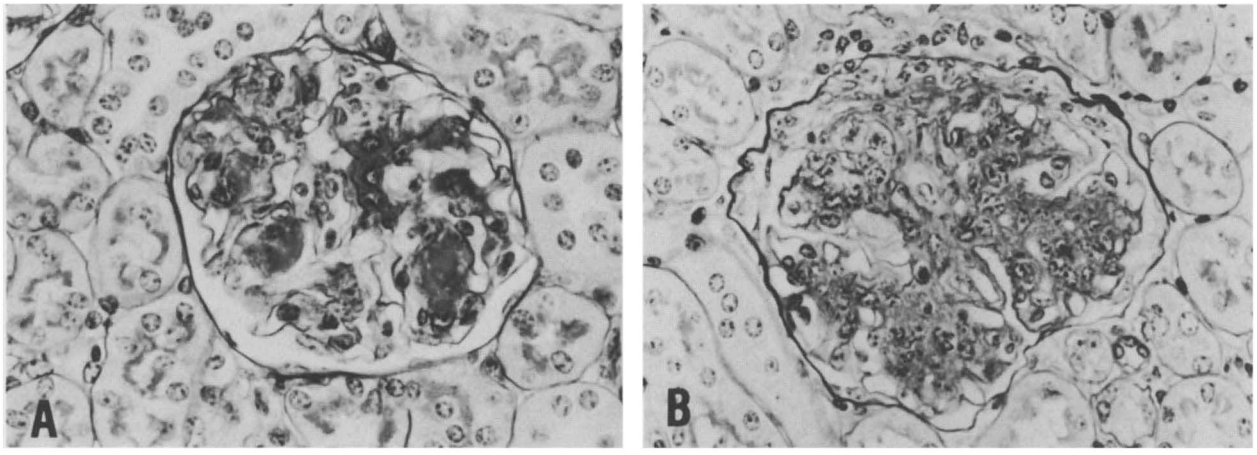

Fig. 1. A. *Prominent PAS-positive deposits in the mesangial area of a glomerulus from a 60 week-old ddY mouse (PAS, ×1570).* **B.** A glomerulus from another 60 week-old mouse. Global expansion of the mesangial area with hypercellularity and the crescent formation at the top of the glomerulus (PAS, ×1360).

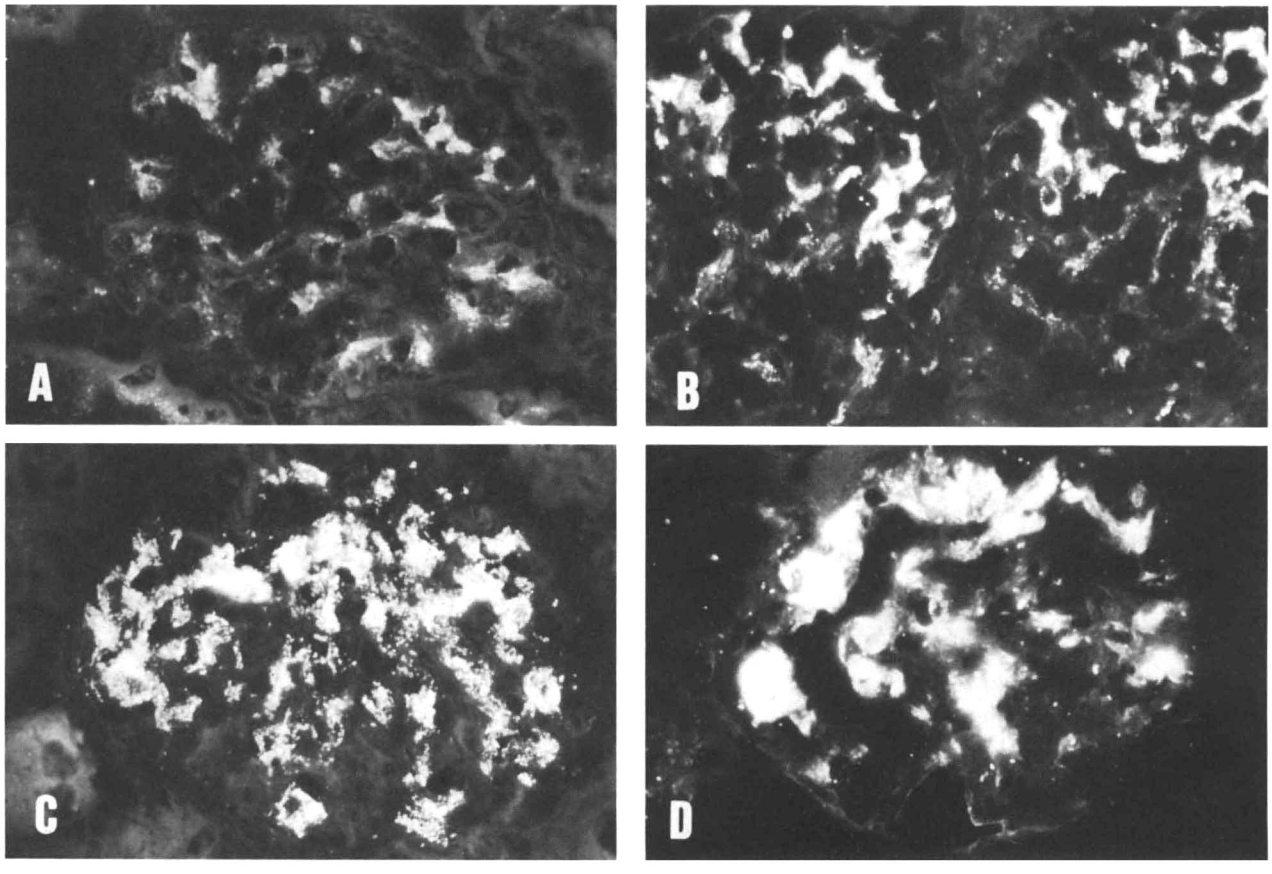

Fig. 2. A-C. *Immunofluorescence findings on the mesangial gp70 deposition; A, B and C correspond to grade 1 (+), grade 2 (++) and grade 3 (+++) deposition, respectively (×1100).* **D.** A characteristic grade 3 (+++) IgA deposition in the mesangial area in a 60 week-old mouse (×1100).

tion and the various extents of gp70 deposition, graded from (+) to (+++), are shown in Figure 2. Electron-dense deposits were observed in the mesangial matrix of these mice using an electron microscope as shown in Figure 3A. Immunoelectron microscopic examinations revealed that at least part of these electron-dense deposits were composed of gp70. It was noted that gp70 was not localized in the cytoplasm of the mesangial cells, but rather, in the mesangial matrix (Fig. 3B).

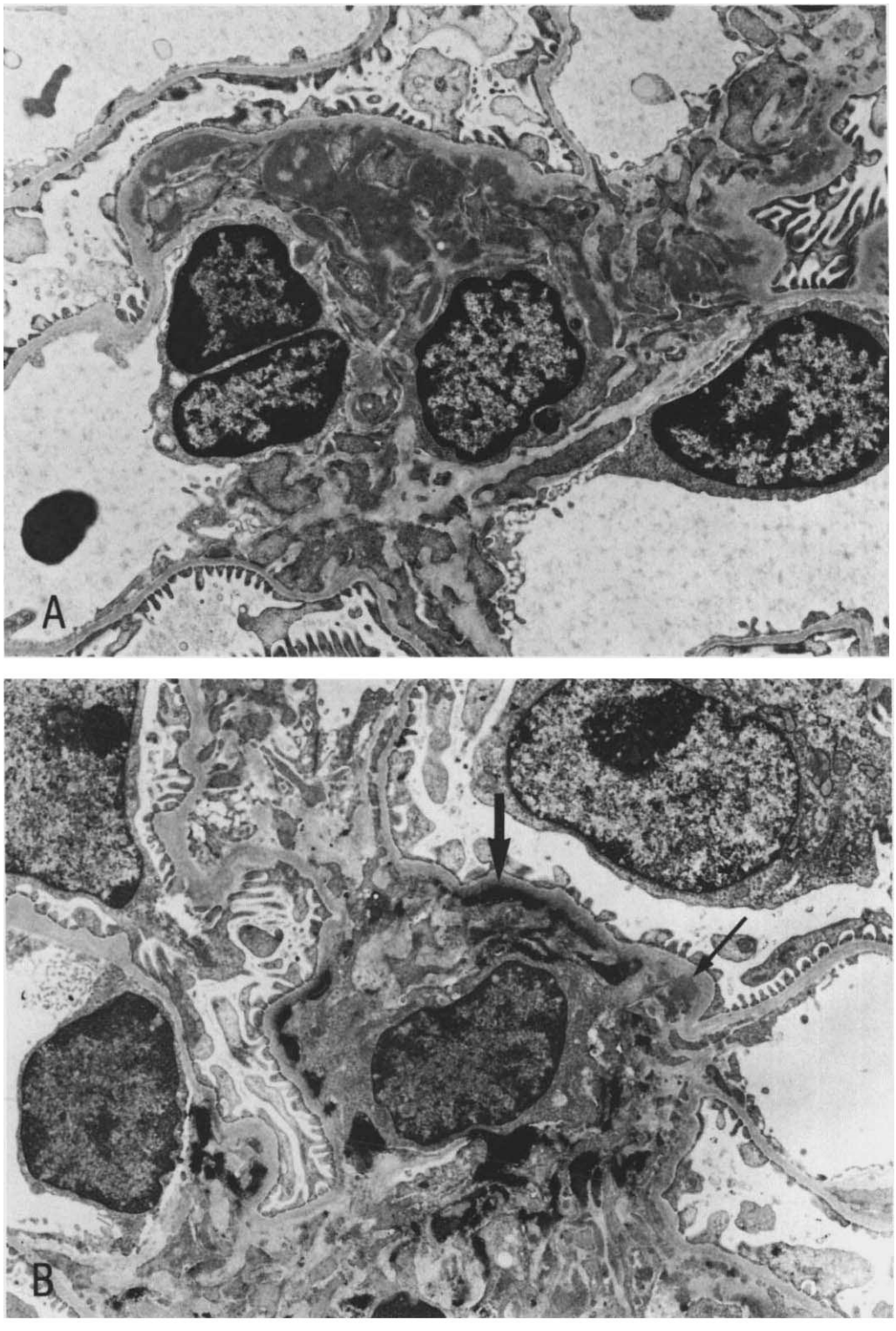

Fig. 3. A. *An electronmicrograph from a 50 week-old mouse (×6700).* Electron dense deposits are demonstrated in the mesangial matrix. **B.** An immunoelectronmicrograph (×6700) poststained with uranyl acetate from the same mouse as in Fig. 3A. Distinct electron dense reaction products of gp70 (thick arrow) can be seen in the mesangial matrix, with nonimmunoreactive electron-dense deposits (thin arrow).

Aging study on glomerular gp70 and immunoglobulins

Deposits of glomerular gp70, IgM, IgG and IgA were compared between groups, consisting of 10 mice each, at different ages, from 16 to 60 weeks old (Table 1). At 16 weeks old, the deposition of gp70 was negative or slightly positive (+). In mice over 24 weeks old, the positivity and intensity increased with

Table 1. Comparison of the grades of glomerular gp70, IgM, IgG and IgA deposition between groups, of 10 ddY mice each, at different ages

Age	gp70				IgM				IgG				IgA			
weeks	−	+	++	+++	−	+	++	+++	−	+	++	+++	−	+	++	+++
16	4	6	0	0	0	7	3	0	3	7	0	0	0	10	0	0
24	1	6	3	0	0	3	5	2	0	7	3	0	0	9	1	0
32	2	7	1	0	0	2	6	2	0	6	3	1	0	6	4	0
40	2	3	3	2	0	4	5	1	0	7	2	1	0	5	5	0
50	0	4	4	2	0	1	5	4	0	0	4	6	0	6	3	1
60	0	5	3	2	0	3	4	3	0	2	4	4	0	2	5	3

Each figure indicates the number of mice exhibiting the corresponding grade of deposition out of a total of 10 mice in each age group.

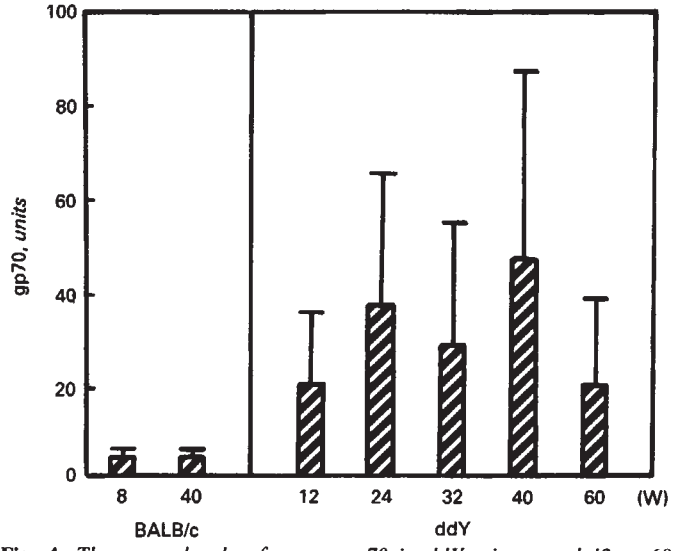

Fig. 4. *The mean levels of serum gp70 in ddY mice, aged 12 to 60 weeks.* For comparison, the results with 8 and 40 week-old BALB/c mice are shown. The bars indicate 1 SD from the mean values.

age. At 32 and 40 weeks, eight out of ten mice were positive. All of the mice over 50 weeks old were positive, and more than 50% of these mice tested moderately (++) or strongly (+++) positive for gp70 deposition. IgM deposition, however, did not appear to be age related. All mice tested positive for IgM deposition, and more than 50% of mice over 24 weeks old showed moderate or strong IgM deposition. On the other hand, the deposition of IgG and IgA tended to increase with aging. In 16 week-old mice, both deposits were negative or slightly positive. Deposits were found to be grade (++) or greater in mice over 24 weeks old. In mice over 40 weeks old, these moderate or strong deposits were observed in higher frequencies.

Serum gp70 levels

Results of the tests on control BALB/c mice showed that serum gp70 levels were low in both 8 and 40 week-old mice, with mean ± SD of 4.3 ± 2.0 units and 4.3 ± 1.5 units, respectively. On the other hand, in ddY mice, gp70 levels varied widely, showing great individual differences at every age tested. There was no apparent age-associated increase of the mean levels. The mean ± SD values at 12, 24, 32, 40 and 60 weeks were 19.9 ± 16.3, 37.4 ± 28.3, 28.4 ± 25.5, 47.0 ± 41.2 and 19.5 ± 18.4 units, respectively (Fig. 4).

Relationship between glomerular gp70 and the immunoglobulins

Fifty mice, aged 24 to 60 weeks old, were analyzed to determine the relationship between the degree of gp70 deposition and those of the immunoglobulins in glomeruli. When the mean scores of IgM, IgG and IgA depositions were compared between groups of mice with different grades (−, +, ++, and +++) of gp70 deposition, it was found that each of these immunoglobulin deposits tended to increase as the grades of the gp70 deposits increased (Fig. 5). This was particularly obvious with IgA deposits: The mean scores of IgA deposition in mice with (−), (+), (++) and (+++) gp70 deposition were 1.0, 1.2, 1.8 and 2.3, respectively.

Comparison between serum levels and glomerular deposits

The serum levels of gp70 and each immunoglobulin were compared between two groups of mice, with weak (− or +) and strong (++ or +++) glomerular deposition of the corresponding proteins (Fig. 6). There were no significant differences in the mean serum levels of gp70 and IgG between the two groups. High serum IgM levels were observed in mice with strong IgM deposition, although this was not statistically significant. Mice with weak IgA deposition generally showed low serum IgA levels with a mean ± SD of 54.3 ± 27.5 mg/dl, while mice with strong IgA deposition tended to show high serum IgA levels with a mean ± SD of 137.1 ± 57.0 mg/ml, with the difference between the two groups being significant ($P < 0.001$).

Absorption of serum gp70 with anti-immunoglobulin columns

Pooled serum samples from 12 and 40 week-old ddY mice were absorbed with anti-mouse IgG and anti-mouse IgA columns. After absorption, the gp70 activities were compared with those of effluent fractions from a control BSA-coupled column (Table 2). The pooled serum from the 12 week-old mice did not show any significant reduction in gp70 activity after absorption with either column. On the other hand, the gp70 activity of the pooled serum from the 40 week-old mice was noticeably reduced after absorption with either column. That is, with the anti-IgG column, the gp70 activity was reduced to 57.9%, while with the anti-IgA column, the activity reduced to 59.5%, compared with that of the BSA-column effluents.

Tissue distribution of gp70

In the immunofluorescence studies, gp70 was detected in frozen sections of various organs from two BALB/c (2 months-old) and each of five ddY mice, aged 12 and 60 weeks (Fig. 7). In the BALB/c mice, gp70 was faintly positive in the bronchial

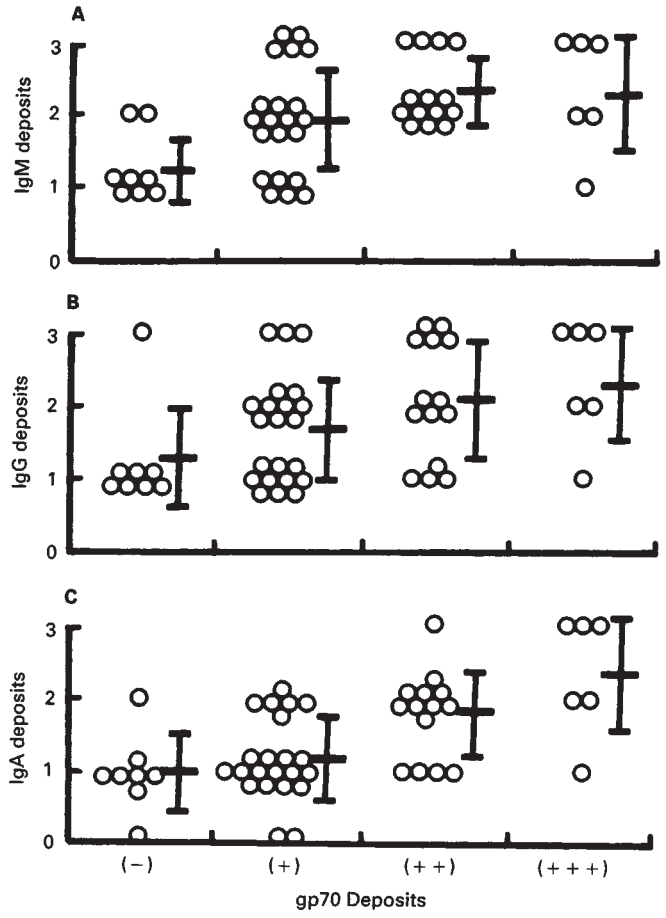

Fig. 5. *Comparison of the grades of glomerular IgM, IgG and IgA deposition in relation to the extent of gp70 deposition.* The bars indicate the means ± 1 SD.

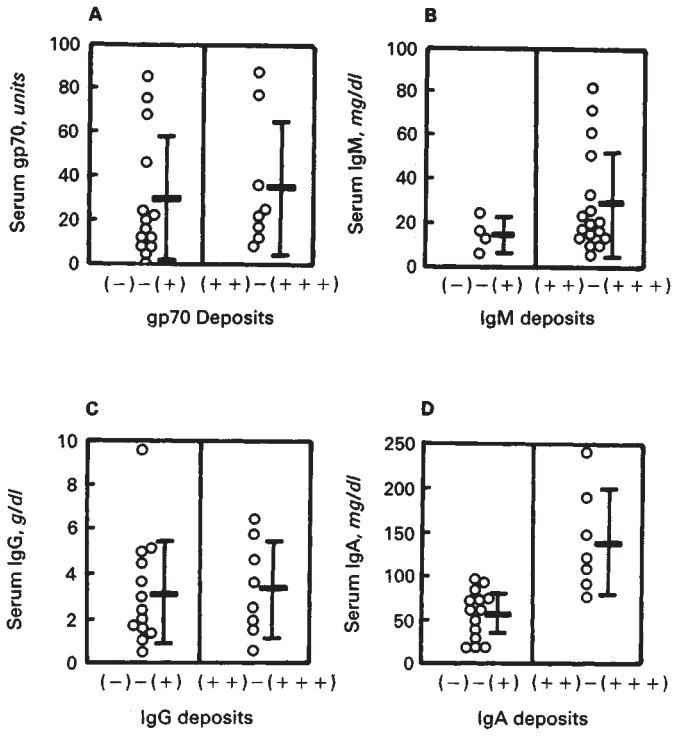

Fig. 6. *Comparison between the serum levels and the grades of glomerular deposition of gp70(A), IgM(B), IgG(C) and IgA(D) in 22 ddY mice, aged 24 to 40 weeks.*

Table 2. Results of the absorption test: Serum gp70 activities with BSA, anti-IgG and anti-IgA columns

Age *weeks*	Absorbed with	Concentration in effluent fractions after absorption		
		IgG *μg/ml*	IgA *μg/ml*	gp70 *units*
12	BSA column	121.1	7.8	52.8
	Anti-IgG column	5.7 (4.7)[a]	10.6 (135.8)	50.0 (94.7)
	Anti-IgA column	116.9 (96.5)	0.7 (8.9)	52.3 (99.5)
40	BSA column	256.4	8.4	94.1
	Anti-IgG column	13.0 (5.1)	10.5 (125.0)	54.5 (57.9)
	Anti-IgA column	207.1 (80.8)	1.0 (11.9)	56.0 (59.5)

[a] In parentheses: The percentage value relative to the BSA-column effluent value

epithelial cells, some renal tubular cells and the acinar and ductal epithelial cells of the salivary gland. In the other organs including lymphoid tissues, liver, heart and intestine, gp70 was not detected. In 12 week-old ddY mice, faint staining in the epithelial cells was also observed as in the BALB/c mice. In addition, various grades of positive findings from faint to moderate, were obtained in the thymus, the spleen, cervical and axillary lymph nodes and Peyer's patches. Of these lymphoid tissues, cytoplasmic staining was observed in scattered cells in the periarterial region of the spleen and in clustered cells in the germinal centers of the spleen and various lymph nodes. Especially in Peyer's patches, almost all cells of the germinal center displayed positive staining. It was noted that some small round cells in the lamina propria of the intestinal mucosa were brightly stained in the ddY mice. In 60 week-old ddY mice, the intensity of these lymphoid cytoplasmic stainings was greater, while the epithelial cells remained faintly or weakly stained. Furthermore, the infiltrated leukemic cells in these older mice were also brightly stained.

Discussion

This study was performed to examine the pathogenic role of gp70, a retroviral envelope glycoprotein, in the development of glomerulonephritis in ddY mice. The results show that gp70 accumulates in glomeruli at least partly forming circulating immune complexes.

The development of mesangial proliferative glomerulonephritis associated with mesangial IgA deposition in ddY mice over 40 weeks old, was first described by Imai et al [27]. Although the renal findings in this study were similar to those described by them, the conspicuous IgG deposition as well as IgA deposition was noted with aging. In addition, we observed a variety of cases, with mild to severe glomerular lesions even forming crescents, which mimic human IgA nephropathy. Regarding tumor formation, malignant lymphoma or leukemic cell infiltration in the various organs was observed in four mice over

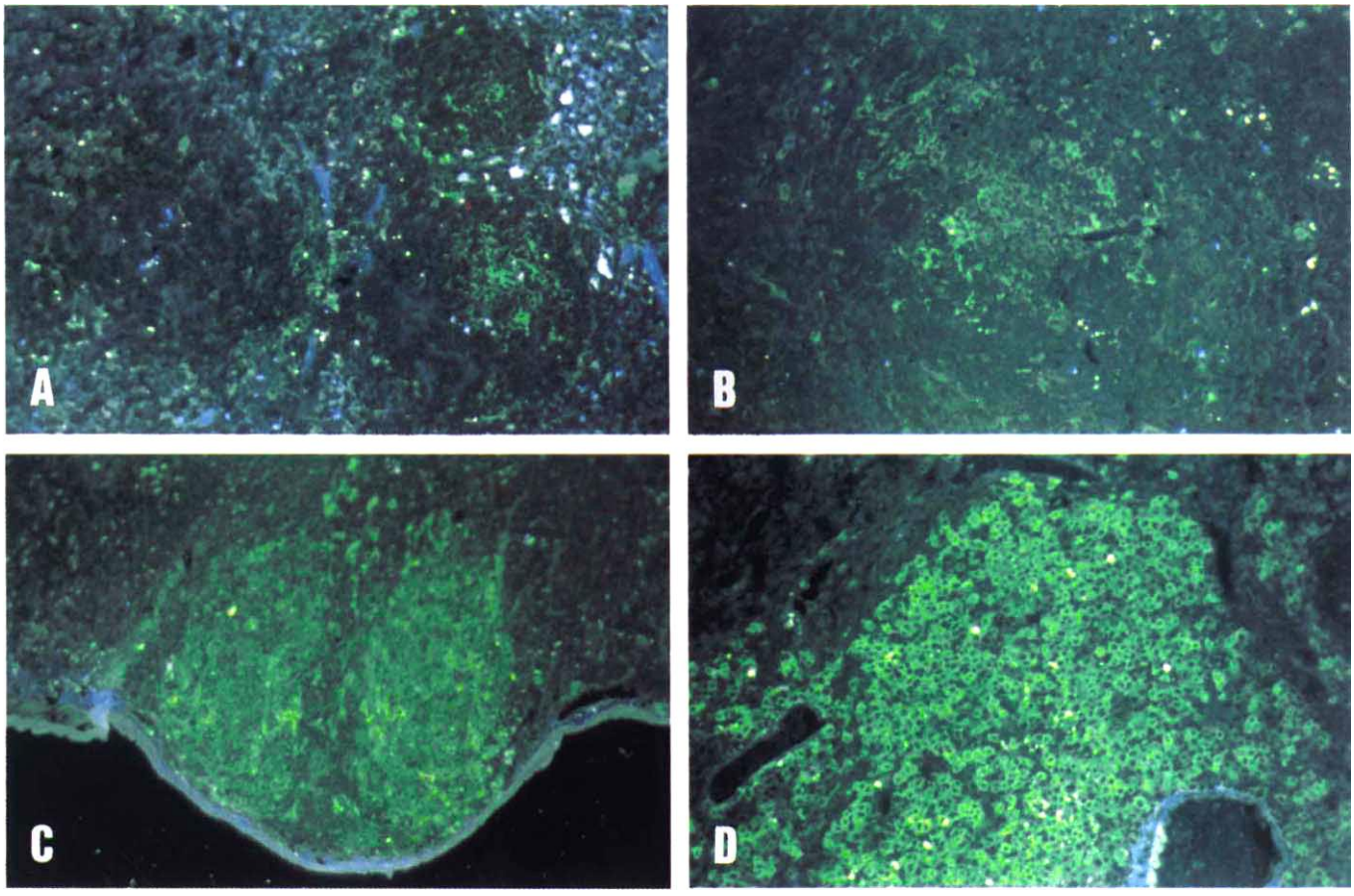

Fig. 7. *Immunofluorescence gp70 staining in the spleen* **(A)**, *the thymus* **(B)**, *Peyer's patch* **(C)** *and the lung* **(D)** *in 60 week-old ddY mice (×264).* **A.** Cells in the germinal center and the periarterial region are positive. **B.** Both clustered and scattered cells in the thymic medulla are stained. **C.** Strongly positive findings are shown in almost all of the germinal center cells. **D.** Conspicuous staining of the infiltrating leukemic cells in the lung.

24 weeks old, out of the total sample of 50 female mice under 60 weeks old, although such tumor formation was not described by the former group. Chino, Sato and Sasaki [28] described the increasing incidences of malignant lymphoma in aging ddY mice. They reported the incidence rates of 2.6, 6.9 and 17.2% in mice aged approximately 60, 80 and 100 weeks, respectively. Notably, they identified C-type retroviral particles in the malignant lymphoma in these mice. These results suggest that the gp70 antigen may be one of the causative factors in the development of the glomerulonephritis in these mice.

Immunofluorescence testing on tissue localization of gp70 in ddY mice yielded positive findings in various lymphoid tissues such as the spleen, the thymus, cervical and axillary lymph nodes and Peyer's patches, with less intensive distributions in various epithelial cells, as was demonstrated in autoimmune New Zealand mice [38]. Although malignant lymphoma or leukemia was observed in only a small fraction of these mice, the positive gp70 findings in the lymphoid cells of all of the ddY mice tested, suggest that the fairly selective expression of an endogenous MuLV genome (gp70) in lymphoid cells may be a common feature of these mice. This characteristic lymphoid distribution of gp70 in mice, aged 12 weeks, with individually different degrees, seem coincident with the early appearance of the wide range of serum gp70 activities. These findings suggest that most of the serum gp70 activities may have been derived from these lymphoid cells in ddY mice.

With regard to the mechanisms of the localization of gp70 in the glomeruli, it seems unlikely that gp70 binds to glomerular mesangium as a free molecule having high affinity for the mesangial tissue, since the serum gp70 levels did not correlate with the amounts of glomerular gp70. It is possible that gp70 may be produced by mesangial cells; however this is also unlikely, because the immunoelectron microscopic examination revealed that gp70 was localized only in the mesangial matrix, and not in the cytoplasm of the mesangial cells. Rather, it seems likely that gp70 circulate as immune complexes which accumulate in the mesangial area. Findings that there is a relationship between the extent of gp70 deposition and that of the IgG and IgA depositions, and that no mice showed gp70 alone in the glomeruli without any immunoglobulin deposition, support the hypothesis mentioned above. The further evidence of the presence of gp70 immune complexes in the sera of mice with glomerulonephritis was obtained using the absorption test with anti-IgG and anti-IgA antibody-coupled affinity column chromatography. Significant absorption rates (approximately 40% with each column) of the gp70 activities were demonstrated with sera of 40 week-old mice which developed characteristic glomerulonephritis. In contrast, almost negligible absorption of

gp70 activities was observed with sera from 12 week-old mice which did not develop the glomerulonephritis. These findings suggest that significant numbers of gp70 molecules circulate as nephritogenic immune complexes in these mice. However, the result of the present absorption study may not directly disclose the presence of gp70-IgA immune complexes nor gp70-IgG immune complexes, because there may be interactions between IgG and IgA antibodies in sera from aged mice developing glomerulonephritis [39].

Recent research indicates that the electric charge is one of the crucial factors in the localization of circulating immune complexes in glomeruli because of the negative charge of the glomerular basement membrane [40, 41]. It has also been reported that the anionic immune complexes tend to accumulate in the mesangial area, whereas the cationic immune complexes tend to localize in the peripheral capillary walls [42, 43]. Assuming that gp70 is an anionic molecule having a pI of 4.6 [44], and that serum IgA antibodies are anionic [45] and the most of serum IgG antibodies are neutral or cationic in electric charge, it seems likely that the totally anionic nature of gp70 immune complexes may give favor for the mesangial localization of these immune complexes, as was observed in this study.

From the viewpoint of the mechanisms of glomerular IgA deposition of these mice, the role of polyclonal B cell activation should be considered, because there was a significant relationship between serum levels of IgA antibodies and the extent of the glomerular IgA deposition, suggesting the direct accumulation of the polyclonally increased IgA in the mesangium. In this context, our preliminary analysis of the qualitative aspects of IgA has shown that IgA in the sera of mature ddY mice were found to be both multimeric and polyclonal (unpublished observation).

Of the various animal models of IgA glomerulonephritis described so far, the ddY mice model is unique because of the spontaneous age-related mesangial IgA and IgG deposition in the background of retroviral infection. Studying this model would help us elucidate the pathogenic role of circulating immune complexes made of the known viral antigen (gp70), and also the fundamental mechanisms of the hyperproduction of IgA antibodies.

Acknowledgments

A part of this work has been presented at the Xth International Congress of Nephrology, London, July 1987. This study was supported by grants from the Ministry of Education, Science and Culture and from the Ministry of Health and Welfare, Japan. Goat anti-Rauscher gp70 antiserum used in this study was a gift from Dr. John S. Cole III, National Cancer Institute, Bethesda, Maryland, USA. The authors thank Prof. Y. Hamashima and Prof. O. Midorikawa for constant supports and thoughtful discussion, Mr. M. Fujioka for technique in electron microscopy, Miss M. Asano and Miss H. Minami for technical assistance.

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References

1. Berger J, Hinglais N: Les depots intercapillaires d'IgA-IgG. *J Urol Nephrol* 74:694–695, 1968
2. Berger J: IgA glomerular deposits in renal disease. *Transplant Proc* 1:939–944, 1969
3. Finlayson G, Alexander R, Juncos L, Schlein E, Teague P, Waldman R, Cade R: Immunoglobulin A glomerulonephritis. A clinicopathologic study. *Lab Invest* 32:140–148, 1975
4. Clarkson AR, Seymour AE, Thompson AJ, Haynes WDG, Chan YL, Jackson B: IgA nephropathy: A syndrome of uniform morphology, diverse clinical features and uncertain prognosis. *Clin Nephrol* 8:459–471, 1977
5. Woodroffe AJ, Gormly AA, McKenzie PE, Wootton AM, Thompson AJ: Immunologic studies in IgA nephropathy. *Kidney Int* 18:366–374, 1980
6. Doi T, Kanatsu K, Sekita K, Yoshida H, Nagai H, Hamashima Y: Circulating immune complexes of IgG, IgA, and IgM classes in various glomerular diseases. *Nephron* 32:335–341, 1982
7. Hall RP, Stachura I, Cason J, Whiteside TL, Lawley TJ: IgA-containing circulating immune complexes in patients with IgA nephropathy. *Am J Med* 74:56–63, 1983
8. Doi T, Kanatsu K, Sekita K, Yoshida H, Nagai H, Hamashima Y: Detection of IgA class circulating immune complexes bound to anti-C3d antibody in patients with IgA nephropathy. *J Immunol Methods* 69:95–104, 1984
9. Hernando P, Egido J, Nicolas R, Sancho J: Clinical significance of polymeric and monomeric IgA complexes in patients with IgA nephropathy. *Am J Kidney Dis* 6:410–416, 1986
10. Lesavre PH, Digeon M, Bach JF: Analysis of circulating IgA and detection of immune complexes in primary IgA nephropathy. *Clin Exp Immunol* 48:61–69, 1982
11. Tomino Y, Sakai H, Endoh M, Suga T, Miura M, Kaneshige H, Nomoto Y: Cross-reactivity of IgA antibodies between renal mesangial areas and nuclei of tonsillar cells in patients with IgA nephropathy. *Clin Exp Immunol* 51:605–610, 1983
12. Bene MC, Faure G, Hurault de Ligny B, Kessler M, Duheille J: Immunoglobulin A nephropathy. Quantitative immunohistomorphometry of the tonsillar plasma cells evidences an inversion of the immunoglobulin A versus immunoglobulin G secreting balance. *J Clin Invest* 71:1342–1347, 1983
13. Sancho J, Egido J, Rivera F, Hernando L: Immune complexes in IgA nephropathy: presence of antibodies against diet antigen and delayed clearance of specific polymeric IgA immune complexes. *Clin Exp Immunol* 54:194–202, 1983
14. Sabatier JC, Genin C, Assenat H, Colon S, Ducret F, Berthoux FC: Mesangial IgA glomerulonephritis in HLA-identical brothers. *Clin Nephrol* 11:35–38, 1979
15. Richman AV, Mahoney JJ, Fuller TJ: Higher prevalance of HLA-B12 in patients with IgA nephropathy. (abstract) *Ann Intern Med* 90:201, 1979
16. Monioliu J, Darnell A, Torras A, Ercirra G, Valles M, Revert L: Familial IgA nephropathy: Report of two cases and brief review of the literature. *Arch Intern Med* 140:1374–1375, 1980
17. Kashiwabara H, Shishido H, Tomura S, Tuchida H, Miyajima T: Strong association between IgA nephropathy and HLA-DR4 antigen. *Kidney Int* 23:377–382, 1982
18. Sakai H, Nomoto Y, Arimori S, Komori K, Inouye H, Tsuji K: Increase of IgA-bearing peripheral blood lymphocytes in families of patients with IgA nephropathy. *Am J Clin Pathol* 72:452–456, 1979
19. Sakai H, Nomoto Y, Arimori S: Decrease of IgA specific suppressor T cell activity in patients with IgA nephropathy. *Clin Exp Immunol* 38:243–248, 1979
20. Egido J, Blasco R, Sancho J, Lozano L, Sanchez Crespo M, Hernando L: Increased rates of polymeric IgA synthesis by circulating lymphoid cells in IgA mesangial glomerulonephritis. *Clin Exp Immunol* 47:309–316, 1982
21. Egido J, Blasco R, Sancho J, Lozano L: T cell dysfunction in IgA nephropathy: specific abnormalities in the regulation of IgA synthesis. *Clin Immunol Immunopathol* 26:201–211, 1983
22. Rifai A, Small PA, Teague PO, Ayoub EM: Experimental IgA nephropathy. *J Exp Med* 150:1161–1173, 1979
23. Isaacs K, Miller F, Lane B: Experimental model for IgA nephropathy. *Clin Immunol Immunopathol* 20:419–426, 1981
24. Emancipator SN, Gallo GR, Lamm ME: Experimental IgA nephropathy induced by oral immunization. *J Exp Med* 157:572–582, 1982
25. Portis JL, Coe JE: Deposition of IgA in renal glomeruli of mink affected with Aleutian disease. *Am J Pathol* 96:227–236, 1979

26. Porter DD, Larsen AE, Porter HG: The pathogenesis of Aleutian disease of mink: In vivo viral replication and the host antibody response to viral antigen. *J Exp Med* 130:575–589, 1969
27. Imai H, Nakamoto Y, Asakura K, Miki K, Yasuda T, Miura AB: Spontaneous glomerular IgA deposition in ddY mice: An animal model of IgA nephritis. *Kidney Int* 27:756–761, 1985
28. Chino F, Sato F, Sasaki S: Retrovirus particles in spontaneously occurring and radiation-induced tumors in ddY mice. *Acta Pathol Jpn* 31:233–247, 1981
29. Yoshiki T, Mellors RC, Strand M, August JT: The viral envelope glycoprotein of murine leukemia virus and the pathogenesis of immune complex glomerulonephritis of New Zealand mice. *J Exp Med* 140:1011–1027, 1974
30. Izui S, MaConahey PJ, Theofilopoulos AN, Dixon FJ: Association of circulating retroviral gp70-anti-gp70 immune complexes with murine systemic lupus erythematosus. *J Exp Med* 149:1099–1116, 1979
31. Maruyama N, Furukawa F, Nakai Y, Sasaki Y, Ohta K, Ozaki S, Hirose S, Shirai T: Genetic studies of autoimmunity in New Zealand mice: IV. contribution of NZB and NZW genes to the spontaneous occurrence of retroviral gp70 immune complexes in (NZB × NZW)F1 hybrid and the correlation to renal disease. *J Immunol* 130:740–746, 1983
32. Yoshiki T, Hayasaka T, Fukatsu R, Shirai T, Itoh T, Ikeda H, Katagiri T: The structural proteins of murine leukemia virus and the pathogenesis of necrotizing arteritis and glomerulonephritis in SL/Ni mice. *J Immunol* 122:1812–1820, 1979
33. Doi T, Kanatsu K, Nagai H, Kohrogi N, Kuwahara T, Hamashima Y: Immunoelectron microscopic studies of IgA nephropathy. *Nephron* 36:246–251, 1984
34. Guesdon JL, Ternynck T, Avrameas S: The use of Avidin-Biotin interaction in immunoenzymatic techniques. *J Histochem Cytochem* 27:1131–1139, 1979
35. Hara I, Izui S, Dixon FJ: Murine serum glycoprotein gp70 behaves as an acute phase reactant. *J Exp Med* 155:345–357, 1982
36. Kennel SJ: Purification of a glycoprotein from mouse ascites fluid by immunoaffinity chromatography which is related to the major glycoprotein of murine leukemia viruses: Immunologic and structural comparison with purified viral glycoproteins. *J Biol Chem* 251: 6197–6204, 1976
37. Izui S, Eisenberg RA, Dixon FJ: IgM rheumatoid factors in mice injected with bacterial lipopolysaccharides. *J Immunol* 122:2096–2102, 1979
38. Lerner RA, Wilson CB, Del Villano BC, McConahey PJ, Dixon FJ: Endogenous oncornaviral gene expression in adult and fetal mice: Quantitative, histologic and physiologic studies of the major viral glycoprotein, gp70. *J Exp Med* 143:151–166, 1976
39. Czerkinsky C, Koopman WJ, Jackson S, Collins JE, Crago SS, Schrohenloher RE, Julian BA, Galla JH, Mestecky J: Circulating immune complexes and immunoglobulin A rheumatoid factor in patients with mesangial immunoglobulin A nephropathies. *J Clin Invest* 77:1931–1938, 1986
40. Rennke HG, Cotran RS, Venkatachalam MV: Role of molecular charge in glomerular permeability: Tracer studies with cationic ferritins. *J Cell Biol* 67:638–646, 1975
41. Kanwar YS, Farquhar MG: Anionic sites in the glomerular basement membrane: In vivo and in vitro localization to the lamina rarae by cationic probes. *J Cell Biol* 81:137–153, 1976
42. Gauthier VJ, Striker GE, Mannik M: Glomerular localization of preformed immune complexes prepared with anionic antibodies or with cationic antigens. *Lab Invest* 50:636–644, 1984
43. Gallo GR, Caulin-Glaser T, Lamm ME: Charge of circulating immune complexes as a factor in glomerular basement membrane localization in mice. *J Clin Invest* 67:1305–1313, 1981
44. Dayer E, Yoshida H, Izui S, Lambert PH: Quantitation of retroviral gp70 antigen, autoantibodies, and immune comlexes in extravascular space in arthritic MRL-lpr/lpr mice. *Arthr Rheum* 30:1274–1282, 1987
45. Monteiro RC, Halbwachs-Mecarelli L, Roque-Barreira MC, Noel LH, Berger J, Lesavre P: Charge and size of mesangial IgA in IgA nephropathy. *Kidney Int* 28:666–671, 1985